EMBRYONIC AND REGENERATIVE DEVELOPMENT IN PLANARIANS.

CHARLES RUSSELL BARDEEN,

Marine Biological Laboratory, Woods Holl, Mass.

The term "regenerative development" may be applied to the formation of a new from a part of a preëxisting individual whereby certain structures are carried over essentially unchanged from parent to daughter. In the following paper regenerative and embryonic development in *Planaria maculata* are compared.

Embryonic Development.

The classical account of the embryonic development of planarians is given in the excellent paper of Iijima, published nearly twenty years ago.[1] Iijima studied more especially the development of *Dendrocœlum lacteum*. The development of *Planaria maculata* seems to be essentially similar. With the early embryonic stages down to the formation of the "embryo-pharynx" we are not at present concerned. It is the transformation of the spherical embryo, filled with yolk cells, into a worm of normal structure and proportions that is of special interest from the standpoint of regenerative development.

At the period when the imbibition of yolk cells begins to be active the planarian embryo is spherical in form. It is surrounded externally by a well-marked membrane formed of greatly distended, flat epithelial cells, shown in section in Fig. 2, *A*. Between this membrane and the yolk cells which fill the central cavity is a reticular substance in which are scattered large cells with nuclei containing one or more nucleoli. These cells are represented by the larger bodies in the body-wall shown in Fig. 2, *A*. One of them is shown in Fig. 4, *a*. The nucleus is immediately surrounded by a granular mass of protoplasm. The reticular substance of the body wall probably represents the

[1] Iijima: "Unters. uber die Bau u. die Entwicklungsgesch. d. Süsswasser-Dendrocœlen (Tricladen)," *Zeitschrift f. Wissenschaftliche Zoölogie*, Vol. XL., 359, 1884.

syncytial "ectoplasm" of these cells. Cell division at this stage is mainly by mitosis. In addition to the cells just described many small nuclei, granular masses, and vacuoles are embedded in the substance of the body wall. These bodies I take to be remnants of yolk cells. Most of the small nuclei show marked evidences of disorganization. Some of the large cells of the body wall lie flattened against the yolk cavity but I can find no evidence at this stage of a differentiation between entoderm and mesoderm cells like that described by Iijima for *Dendrocœlum*. The embryo-pharynx corresponds closely in structure to that described by Iijima.

As the embryo becomes distended by taking in yolk cells the body wall becomes thinner and the character of the cells lying in the reticular substance is altered. The nucleus becomes more densely granular and division takes place mainly by amitosis (see Fig. 4, *b*). The protoplasm of these cells varies in granulation and in staining power, but I have been able to distinguish no clearly defined classes of cells. Rapid cell division gives rise to a smaller variety of cell shown in Fig. 4, *c* and *d*. These cells exhibit indications of amœboid movement, as shown in Fig. 4, *d*. In these smaller cells mitotic division prevails, though amitosis is also present.

The hitherto spherical embryo now becomes flattened dorsoventrally and elongated in the antero-posterior axis. This change in form is accompanied by a more rapid cell-multiplication in the ventral than in the dorsal body wall. The most rapid cell-multiplication takes place on the postero-ventral wall just below the embryo-pharynx (see Fig. 2, *B*). It is from this mass of cells that the permanent pharynx is developed. Iijima says that in *D. lacteum* the permanent pharynx is developed in the region of the embryo-pharynx after the disappearance of the latter. The relations between embryonic and permanent pharynxes in *Planaria maculata* were first discovered by Winterton Curtis and are described by him in an article shortly to appear in the *Proceedings* of the Boston Society of Natural History.

At the period of the formation of the anlage of the permanent pharynx tissue-differentiation becomes active. Before considering this tissue-differentiation it may be best, however, first to go over in brief outline the assumption by the embryo of the form characteristic of the adult.

In Fig. 1 are shown four stages in the development of the embryo. These figures were sketched by means of a camera lucida from living specimens. The outline of the intestinal cavity, as it appeared by transmitted light, is shown by serrated lines. In *D* the septa extending into the intestinal cavity are represented more highly developed than is common at this stage.

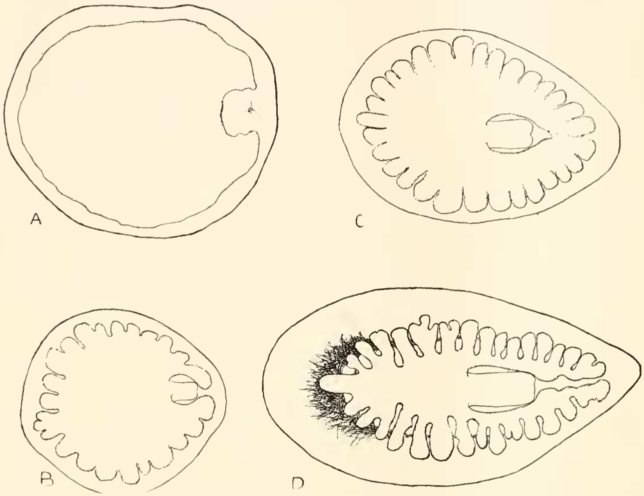


FIG. 1. Various stages in the development of the embryo. Magnification, 57 diameters.

A. Dorsal view of an embryo in which the anlage of the permanent pharynx is being developed. At the right the opening into the embryo-pharynx is shown.

B. Ventral view of an embryo in which the pharynx has begun to assume definite form.

C. Ventral view of an older embryo in which the two posterior extensions of the intestinal cavity have united behind the pharyngeal pocket.

D. Dorsal view of an embryo at the period when the central nervous system has assumed definite outlines.

This was done so as to expose the nervous system. The latter was reconstructed from sections made from the embryo. In *A* the opening into the embryo-pharynx is shown at the right. In

B the permanent pharynx is shown near the posterior end of the piece. This individual seems to have been a small one at the time of metamorphosis. In *C* and *D* the development of the tail posterior to the pharyngeal region is shown. There is a temporary anastomosis of the posterior rami behind the pharyngeal pocket. This anastomosis later disappears, as shown in Fig. 1, *D*. The head is meanwhile developed, as shown in the figures, anterior to the primitive yolk cavity.

The relations of the head, pharyneal and tail regions to the primitive yolk cavity of the embryo are also represented in Fig. 2. In *B* the anlage of the permanent pharynx is shown. The

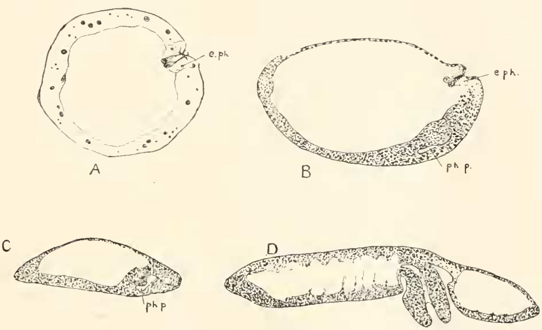


FIG. 2. Sagittal sections in the median line of embryos of various stages of development. Magnification, 57 diameters.

A. Section through an embryo which is becoming filled with yolk cells.

B. Section through an embryo in which both the embryo-pharynx and the anlage of the permanent pharynx are seen.

C. Section through an embryo in which the two posterior extensions of the intestinal cavity are uniting behind the pharyngeal pocket.

D. Section through an embryo in which the tail region is well developed and the anlage of the head is well marked. Owing to the longitudinal contraction caused by the killing fluid the pharynx has been partly forced out from the pharyngeal pocket. *e.ph.*, Embryo pharynx. *ph.p.*, Pharyngeal pocket.

base of the pharynx extends obliquely from the region of the embryo-pharynx to the ventral wall. In *C* the base of the pharynx still extends obliquely from the dorsal to the ventral walls. The embryo-pharynx has disappeared. A tail region posterior to the pharynx and a head region anterior to the yolk cavity are distinctly developed. In *D* the assumption of adult condition is much more advanced. The base of the pharynx now extends nearly perpendicular from the dorsal to the ventral body wall. Head and tail regions are well developed.

The first indications of a definite nervous system appear in the region of the head at about the stage of development shown in Fig. 1, *C*. The outlines of the nervous system are, however, at this period too indefinite for satisfactory illustration. There are apparently bilateral anlages soon united by a commissure. The lateral cords gradually extend from the region of the brain towards the tail. At the period shown in Fig. 1, *D*, the central nervous system has the form there shown somewhat diagrammatically.

The median nerves and commissures and the lateral nerves probably arise by outgrowth from the central nervous system. Nothing is known of the development of the peripheral plexus.

Reproductive organs are developed only after the worm has advanced far in development. Curtis, (*op. cit.*) has followed the development of the reproductive organs in *Planaria maculata*. I shall therefore attempt no description of the process in this place.

Histogenesis in the Embryo.

The development of the various tissues during the formation of the structures characteristic of the adult is difficult to follow. For the sake of making a comparative study of the size and structure of the various cells at different stages of development I have used a uniform method of technique. The organisms were first anæsthetized with chloretone [1] to cause relaxation and to prevent the pre-mortem contraction which killing fluids are apt to stimulate. They were then fixed in a one-half saturated solution of corrosive sublimate to which acetic acid was added, carried through alcohol solutions of increasing strengths up to absolute alcohol, and thence into xylol. The tissues were embedded in paraffine, cut into sections $3\frac{1}{3}$ to $6\frac{2}{3}\mu$ thick, and stained in dilute Delafield's hæmatoxylin, followed by a solution of Congo red in water. A little Congo red was also added to the alcohol used in dehydration. The figures have been drawn to a fixed scale by means of the camera lucida.

[1] A term applied commercially to chloroform-acetone.

The surface epithelium is the simplest of the tissues to follow, owing to the sharp line of demarcation existing between it and the underlying tissues. Up to the time of the formation of the anlage of the permanent pharynx the surface membrane is beset but sparsely with cells. Fig. 3, *a*, shows one of these cells in cross-section. During the period of assumption of adult structure by the embryo these cells multiply rapidly by amitosis, as shown in Fig. 3, *b*. The cells increase in thickness, Fig. 3, *d*.

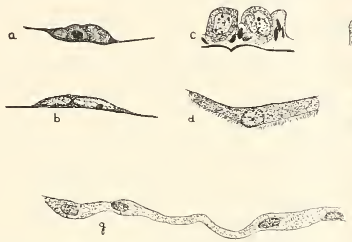


FIG. 3. Sections illustrating various stages in the development of the surface epithelium. Magnification, 540 diameters.

a. From the embryo shown in Fig. 1, *A*.

b. From an embryo in which the pharyngeal anlage is being formed.

c. Dorsal epithelium near the pharyngeal region of an embryo in which the nervous system is beginning to appear.

d. Epithelium from the ventral surface of the tail region of the same embryo.

e. Dorsal epithelial cells from an adult individual.

f. Ventral epithelial cell from an adult individual.

g. Epithelium covering a cut surface of an adult individual twelve hours after injury.

In *b* and *c* evidences of direct nuclear division may be seen.

Finally the membrane becomes covered externally with cells so crowded together that the long axis of each cell lies at right angles to the surface of the body, Fig. 3, *c*. This crowding first takes place near the center of the dorsal and ventral surfaces, where bodily extension is least rapid, and only later at the growing extremities of the worm (compare *c* and *d*, Fig. 3). The dorsal epithelium at no time is ciliated (Fig. 3, *c*), the ventral epithelium acquires cilia at an early stage of development (Fig. 3, *d*). Although it is commonly stated in the text-books that fresh-water planarians are completely covered with cilia when young, I have seen no specimens of *Planaria maculata* at any stage of development in which the dorsal epithelium was ciliated. For the sake of comparison a dorsal and a ventral epithelial cell from an adult individual are shown in *e* and *f* of Fig. 3. These seem to be slightly smaller than the embryo cells. Although the possibility cannot be excluded that cells are added to the surface epithelium from the parenchyma, I have found no evidence of this.

The cells of the parenchyma offer a most difficult and uncertain subject for study. Cells of the type shown in Fig. 4, *c*, are

FIG. 4. Sections illustrating the development of the parenchyma. Magnification, 540 diameters.

a. One of the cells surrounding the yolk cavity of the embryo shown in Fig. 1, *A*.

b. Corresponding cell of a slightly older embryo.

c. Type of cell prevailing in the parenchyma of an embryo immediately before the stage when the pharyngeal anlage appears.

d. Similar cell showing evidence of amœboid movement.

e. Cells illustrating the reduction in size which takes place during the tissue differentiation that occurs at the time when the pharyngeal anlage appears.

f. Closely packed cells seen in the anlage of the permanent pharynx.

g. Connective-tissue cells of body wall during this stage.

h. Early rhabdite cell.

i. Connective-tissue cells of adult individual.

j. Large parenchymal cell in resting stage.

k, *l*. Large parenchymal cells undergoing division.

m. Cells from anlage of head thirty-eight hours after isolation of the piece.

those most commonly met with at the stage preceding the development of the permanent pharynx. In certain regions, such as the pharyngeal anlage, these cells may be very closely packed together (Fig. 4, *f*), but if we assume them to form a syncytium we must consider it a syncytium in which the cell territories are fairly well marked. The individual cells may be irregular in shape or distinctly elongated. From cells of this character smaller cells with smaller nuclei arise and form a syncytium of branched cells (Fig. 4, *g*). The connective tissue of the adult is derived from cells of this nature (Fig. 4, *i*), fibrils in the processes connecting the cells becoming ever more distinct. In addition to these fixed connective-tissue cells there exist in the parenchyma of the adult several other types of cells. Some of these, with dark protoplasm, large nuclei, and well-marked nucleoli lying in clear areas, seem to be the direct, undifferentiated descendants of the embryonic cells of the type shown in Fig. 4, *e*. These cells divide by mitosis (Fig. 4, *k* and *l*) and seem to be the chief factor in the regeneration of new parenchyma. Other cells with lighter nuclei and protoplasm the granules of which stain deeply in "acid" dyes may be leucocytes, as Miss Stevens has aptly suggested.[1] Cells of this nature are seen at an early stage and apparently are derived from cells of the type shown in Fig. 4, *e*. Rhabdite cells begin to be differentiated even before the period when the epithelium assumes a columnar structure, but while this latter process is taking place the rhabdite cells ap proach the basement-membrane and rhabdites are passed out into the intercellular areas. In Fig. 3, *c*, the depression in the membrane indicates the area through which rhabdites have been passed. I have seen no clear indications of the passage of the rhabdite cells themselves through the membrane. Mucus cells and the gland cells belonging to the intestinal system arise at a comparatively late stage. I have not been able to trace their origin from any specific cells.

When lining surfaces like the pharyngeal pocket the parenchyma cells assume an epithelial character. These cells have an origin similar to that of the connective-tissue cells.

Intestinal cells become clearly evident at the time when the embryo assumes an ovoid form. They are at first sparingly scattered, but at the time when septa extend into the yolk-cavity so as to give rise to the permanent form of the intestinal system, they begin to multiply by indirect division (Fig. 5, *a*). As in the case of the surface epithelium, this cell multiplication finally gives rise to cells extending away from the base on which they rest

[1] "Notes on Regeneration in *Planaria lugubris*," *Archiv f. Entwickelungsmechanik der Organismen*, XIII., p. 410, 1901.

(Fig. 5, *b*). As pointed out by Iijima, however, this last process is slow, and it is only at a comparatively late period in development that the intestinal epithelium assumes the structure characteristic of the adult. Whether the intestinal epithelium is differentiated at a very early period and then preserves its specificity, like the surface epithelium, or is added to by constant additions from the parenchyma, it is difficult to decide. The former view seems to me perhaps more probable. It must be stated in

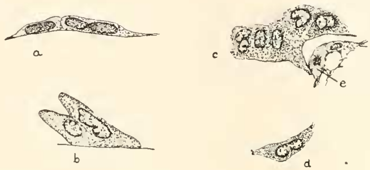


FIG. 5. Sections illustrating the development of the intestinal epithelium. Magnification, 540 diameters.

a. Flat cells seen in embryos at the time of the development of the permanent pharynx.

b. Cells beginning to project toward the lumen of the intestine in an embryo in which the nervous system has just become clearly visible.

c, *d*. Intestinal cells undergoing direct division during regeneration in the adult.

e. Cells bordering on the intestines. In one cell a mitotic figure is visible.

this connection, however, that the parenchyma cells bordering on the intestines show active proliferation by mitosis during embryonic development as well as during regeneration (see Fig. 5, *e*).

Myoblasts become numerous at about the period of the formation of the anlage of the permanent pharynx (Fig. 6, *a*). They are very large cells and apparently are derived from cells of the type shown in Fig. 4, *c*, many of which have more vesicular nuclei than there shown. The myoblasts seem to divide by indirect division. From those lying next the basement membrane of the epithelium is derived a syncytium, a portion of which is shown in Fig. 6, *b*. In this the surface musculature is developed. It is probable that a separate syncytial sheet exists for each layer of musculature. Other myoblasts give rise to the muscle fibers which run through the parenchyma in various directions.

Blochmann,[1] who studied the tissues of cestodes by means of the methylen-blue and silver impregnation methods, describes as "Sommer-Landois cells" large cells lying below the surface musculature and sending out branches connected on the one hand with the fibers of the surface musculature and on the other hand extending into the peripheral nerve plexus. The muscle fibers he

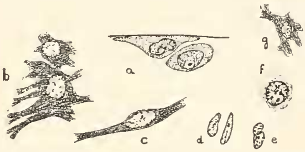


FIG. 6. Sections illustrating the development of the musculature. Magnification, 540 diameters.

a. Myoblasts in an embryo in which the pharyngeal anlage is being formed.
b. Developing circular musculature at a later stage.
c. Developing parenchymal muscle cell.
d, *e*. Small nuclei lying between the muscle fibers of an adult individual.
f. Large cell occupying a corresponding position.
g. Developing circular musculature during regeneration.

considers outgrowths of processes of these cells. In *Planaria maculata* large cells with vesicular nuclei, resembling in size and form the myoblasts of the embryo, may be seen lying below the surface musculature. Whether or not these represent Sommer-Landois cells which have migrated from the syncytial musculature after the formation of the latter cannot be decided by methods which I have employed. Monti has described cells of the Sommer-Landois type in planarians.[2]

It is uncertain whether the musculature of the pharynx arises from the cells potentially differentiated at the time of the formation of the pharyngeal anlage.

Of all the tissues the nervous tissues are the most difficult to follow in these animals. Only by special technical methods can this be satisfactorily done and yet the planarian tissues seem very refractory to the ordinary impregnation methods. Blochmann (*op. cit.*) used these successfully on cestodes and gives an inter-

[1] "Ueber freie Nervenendigungen und Sinneszellen der Bandwürmern," *Biologisches Centralblatt*, XV., 14, 1895.

[2] *Archives Italiennes de Biologie*, Vol. XXVII., p. 15, 1897.

esting description of the elements of the peripheral nerve plexus. In this many nerve cells are found. Monti[1] has stained by the Golgi method and described unipolar, bipolar and multipolar cells lying both in and about the brain and nerve cords and in the periphery next the musculature but she has not followed their development. Bergendal[2] found in the central nervous system of land planarians large ganglion cells, with very large nuclei which stain lightly.

I have made no extended attempt to stain the nervous system of planarians by special methods. In and about the nerve-cords and brain are scattered nuclei that stain very lightly and are much larger than the nuclei of the fixed connective-tissue cells (see Fig. 7, *e*, and compare with Fig. 4, *i*). These I have taken to be the nuclei of nerve cells. The cell-body is usually difficult to follow, but at times this may be seen sending off one or more

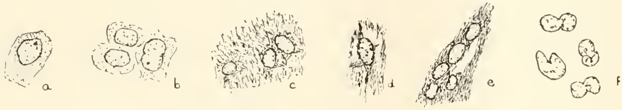


FIG. 7. Sections illustrating the development of the nervous system. Magnification, 540 diameters.

a. Large cell seen in the anterior ventral region of an embryo before the anlage of the pharynx has appeared.

b. Similar cells after the anlage has appeared.

c. Early stage in the development of nerve fibers.

d. Nerve cells in an adult individual.

e. Row of nerve cells seen in a regenerating nerve cord.

f. Nuclei of nerve cells undergoing direct division during regeneration of a new brain.

processes. At the stage of development in the embryo immediately preceding the formation of the permanent pharynx a few large, palely staining cells may be seen in the anterior portion of the ventral body wall. A cell of this kind is shown in Fig. 7, *a*. I have not found these cells elsewhere in these embryos and think it possible that they represent neuroblasts. I have tried to find them in earlier stages of development without success. They

[1] "Sur le system nerveux des Dendroceles d'eau douce," *Archiv. Ital. de Biologie*, Vol. XXVII., 15, 1897.

[2] *Zoölogischer Anzeiger*, X., 218–224, 1887.

do not seem at this period to arise from the surface epithelium. At the time of the formation of the anlage of the permanent pharynx, cells like these in a corresponding region may be seen multiplying by direct division (Fig. 7, *b*). When the brain anlages first become clear, owing to the formation of nerve fibers, similar cells may be seen lying among the fibers (Fig. 7, *c*). In the nerve cords, as they grow toward the tail region, corresponding nuclei may be seen, often in columns and often appearing to divide by direct division. It seems possible, therefore, that the nerve cells of the central nerve system of these planarians may arise by direct division from neuroblasts which are differentiated in the anterior ventral wall of the embryo at a comparatively early period, and that the other cells of the central nervous system may arise by direct division from these.

The cells of the peripheral nerve plexus may arise, however, in the periphery and not from those of the central nervous system. In the adult many very pale nuclei may be seen scattered here and there below the musculature. These nuclei resemble those of the cells found within the central nervous system.

During embryonic development, therefore, we find that the surface epithelial cells are derived by direct division from the preexisting ectoderm cells. The intestinal cells certainly, and also probably the nerve cells, multiply by indirect division. Whether additions are made from parenchyma cells after the primary period of differentiation is uncertain. The early myoblasts multiply partly, at least, by indirect division. The period at which the differentiation of myoblasts from the parenchyma ceases is undetermined. Branched, anastomosing connective-tissue cells, "leucocytes," rhabdite cells and gland cells are differentiated in the parenchyma but certain cells retain a size and form characteristic of an early stage of embryonic development.

Regeneration in Embryos.

When removed from the egg-capsule embryos of the stages represented in Fig. 1, *A* and *B*, and younger ones live but a short time in water. Twenty-four hours is the longest I have been able to keep them alive. Embryos of the stage shown in Fig.

1, *C*, may, with care, be kept alive until adult form is assumed; but if mechanically injured they do not long survive and pieces removed from them will not live. If embryos of the stage shown in Fig. 1, *D*, be cut in a region immediately anterior to the pharynx both anterior and posterior pieces may sometimes be kept alive. The anterior piece will develop a new pharynx, but the posterior piece will not develop a new head, although it may otherwise undergo full development, including the assumption of the pigmentation characteristic of the adult. It seems probable, although it cannot be taken as proved, that the failure of the posterior piece to give rise to a new head is due to the absence in the piece of well-formed nerve cords. If the cut be made near the head or in older embryos a new head may be regenerated.

In *Dendrocœlum lacteum*[1] F. R. Lillie found that a new tail may be formed by outgrowth from any transverse level behind the region immediately back of the eyes, but a head only on a cut surface made in the anterior third of the body. These results have been confirmed by E. Schultze[2] and by me. Lillie ascribes the result to the cephalization of the nervous system in *D. lacteum*. It is interesting to note that at the period when the central nervous system is "cephalized" in *Planaria maculata* similar conditions prevail.

Regenerative Development.

For the sake of comparing embryonic with regenerative development we may consider briefly the regeneration of new individuals from cross pieces taken from in front of the pharynx, from tail pieces and from slips removed from a region lateral to the median line.

Cross Pieces.—There is a close similarity between the development of a new individual from a cross piece cut from in front of the pharynx and development in the embryo. In the cross piece the pharynx develops on the posterior ventral surface, with relations to the axial gut similar to that borne by the pharynx to the yolk cavity in the embryo (see Fig. 8). The head and tail re-

[1] "Notes on Regeneration and Regulation in Planarians," *American Journal of Physiology*, Vol. VI., p. 128, 1901.

[2] "Aus dem Gebiete der Regeneration bei Turbellarien," *Zeitschrift für wissenschaftliche Zoölogie*, Vol. LXXII., p. 1, 1902.

gions are likewise developed in a manner resembling the embryonic.

If the cut surface on which the new head is developed be at right angles to the long axis of the parent worm the new head will be developed symmetrically about the anterior extremity of the axial gut, just as it is developed symmetrically about the anterior extremity of the yolk-cavity in the embryo. If, however, the cut be made oblique, the head may arise some distance anterior and lateral to the region where the old axial gut projects on the cut surface. Morgan first described this phenomenon.

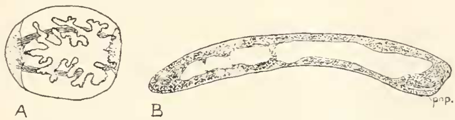


FIG. 8. To illustrate the development of a new individual from a cross piece 48 hours after removal. Magnification, 57 diameters.

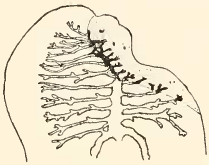
A. Individual as seen from the dorsal surface. The intestinal system was sketched from the living individual, the nervous system was reconstructed from sections.

B. Sagittal section in the median line from the same individual. *ph.p.*, Pharyngeal pocket.

Fig. 9 shows a good example from *Dendrocœlum lacteum.* The median ends of the lateral intestinal branches lying in the piece anterior to the extremity of the old axial gut and separated from the latter by the cut have become so anastomosed as to form an extension of the old axial gut. About the anterior extremity of this the new head is being symmetrically differentiated. In the further growth of the piece new tissue is rapidly formed to the right of the axial gut and about its extremity until the worm is restored to normal proportions.

Tail Pieces. — In tail pieces regeneration of the new head takes place symmetrically about a branch extending forward from the area of fusion of the two posterior rami of the original piece. In all of the instances which I have examined this fusion has taken place before differentiation of a head has really begun. The process of head formation resembles essentially that described as taking place on the anterior surface of the cross piece taken from in front of the pharyngeal region. The formation of the pharynx is the most puzzling process in the regeneration of these tail pieces.

The region of pharynx formation depends upon the condition of the piece and the region of the cut. In individuals not sexually mature if the cut be made posterior to the old pharynx the new pharynx will develop slightly anterior to the center of the piece (Fig. 11, *B*). I have followed the development of a large number of such specimens during a cool spell when the regenerative processes were somewhat slow, and the successive stages in regeneration therefore well marked. The following notes in-



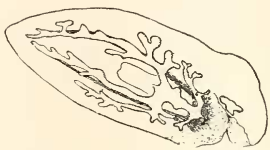


FIG. 9. To illustrate the formation of a new axial gut lateral to the axis of the original gut, after oblique section; *D. lacteum*. Magnification, 17 diameters. The regenerated intestinal branches are shown in black.

FIG. 10. To illustrate the development of a head symmetrically about the end of the axial gut and posterior to an anterior lateral slip which contained no nervous system. Magnification, 17 diameters.

dicate these stages. The number of hours given in each instance shows the length of time elapsing after isolation of the pieces:

Eighteen hours. The cut surface is covered with epithelium and there is some accumulation of parenchyma cells near it.

Thirty-eight hours. Further accumulation of cells near the cut surface.

Sixty-six hours. Anastomosis of the posterior intestinal rami in the vicinity of the cut surface (Fig. 11, *A*). Advance of nerve fibers into the head anlage. In some specimens there is a slight accumulation of cells in the vicinity of the future pharynx.

Ninety hours. The accumulation of cells in the pharyngeal anlage is more marked. Between the region of anastomosis of the posterior rami and the pharyngeal anlage new intestinal branches are being formed. The head anlage has advanced in development.

One hundred and fourteen hours. Considerable anastomosis between the intestinal branches last mentioned. In some specimens a new axial intestine had thus been formed between the anlage of the head and that of the pharynx (Fig. 11, *B*). The pharynx is beginning to be differentiated. The head is much advanced in development but the chief commissure of the brain is not completely formed.

One hundred and thirty-eight hours. The axial intestine and pharynx are well formed. The chief commissure of the brain is well formed and the eyes have appeared.

Subsequently the axial intestine formed between the two original posterior rami becomes greatly developed at the expense of the latter.

Sometimes the process is more simple than that described above, the anterior portion of one or the other of the posterior rami becoming converted directly into an axial gut; or fusion of two rami may be more complete, as in the instance described in my previous paper.[1] Miss Stevens (*op. cit.*) states that the accumulation of cells for the pharyngeal anlage may begin in *P. lugubris* before intestinal anastomosis takes place. We must admit, therefore, that in instances of this kind the pharyngeal anlage is not formed in response to stimuli arising from a definite axial intestinal cavity, as seems to be the case in the embryo and in pieces cut anterior to the pharynx, but rather upon the relations borne by a given area of the worm to the intestinal system as a whole.

In sexually mature worms cut immediately anterior to the genital apparatus the pharynx is formed much further anterior than in sexually immature specimens. The reproductive organs undergo retrograde metamorphosis. Destruction of the genitals begins at the periphery of these organs where the intestines border upon them and extends towards the center (see Fig. 11, *C*). The process is a complex one which I shall not attempt to describe in this place. The ducts and glands, including the testicles, also become disorganized, the last to disappear being those near the posterior end of the animal. As a result of the breaking down of these organs the neighboring tissues of the planarian

[1] "On the Physiology of Regeneration in *Planaria maculata* with Especial Reference to the Phenomena of Regeneration," *American Journal of Physiology*, Vol. V., p. 1, 1901.

become filled with bits of disorganized tissue. Most of this eventually finds its way into the intestines which become thereby so distended that in many places the lining epithelium is as thin as that found during embryonic development. Stevens (*op. cit.*) has described a similar process in *P. lugubris.* The pharyngeal anlage is formed in the median line immediately anterior to the degenerating sexual apparatus and posterior to the center of the anastomosis between the posterior rami (Fig. 11, *c*).

If the cut isolating the tail piece pass through the pharyngeal pocket the new pharynx will develop at the anterior end of the

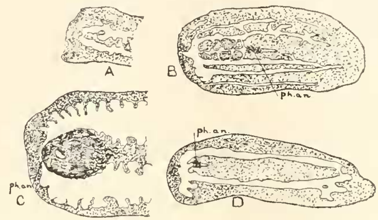


FIG. 11. To illustrate the development of new individuals from tail pieces. Magnification, 17 diameters.

A. Intestinal anastomosis, sixty-six hours after isolation of the piece.

B. Intestinal anastomosis ninety hours after isolation of the piece. The pharyngeal anlage is indicated, *ph.an.*

A and *B* are from individuals not sexually mature.

C. Piece from an individual sexually mature. The reproductive organs are undergoing degeneration.

D. Tail piece isolated by a section passing through the pharyngeal pocket.

pocket. If a portion of the tip of the old pharynx be retained in the chamber this tip will be utilized to form the end of the new pharynx.[1]

Lateral Slips.—In lateral slips isolated by a cut lateral to the region of a lateral nerve cord no specific regeneration takes place. This, at least, is the result I have obtained not only in *P. maculata* but also in *P. lugubris*, *Dendrocœlum lacteum* and *Phagocata gracilis.* If a part of the lateral cord be present in such a

[1] The process of retrograde metamorphosis of the retained portion of the pharynx which I previously described (*op. cit.*, p. 34) is the exception, not the rule. If a portion either of the base or the tip of the pharynx be retained in the piece it will usually be utilized in the regeneration of the new pharynx.

slip a new head will be regenerated, and if a fairly considerable amount of the intestinal apparatus be contained in the slip, a new pharynx will be formed. The intestinal apparatus, if it originally consists of isolated parts, tends to become anastomosed into a whole. A new head is formed about that portion of the intestinal system which projects on the cut surface in the neighborhood of the most developed portion of the nerve-cord (see Fig. 12, *A*). The new pharynx is formed posterior to the region of least bodily compression, polarity being understood to be determined by the nervous system. If the lateral slip be cut so that

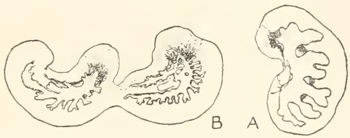


FIG. 12. To illustrate the development of an individual from a lateral slip. Magnification, 17 diameters.

A. Piece isolated from the region lateral to the pharyngeal pocket.

B. Two individuals developing from a lateral slip from the center of which a portion of the nerve cord was removed.

two or more regions will be formed in which the nervous and intestinal apparatus of each section will be isolated from that of the neighboring section, double or even triple individuals may be formed. Fig 12, *B*, indicates a double individual of this kind.

Having thus briefly considered the regeneration of new individuals from these typical isolated pieces, we may now take up, first, the question of histogenesis during regeneration and, second, that of the conditions determining the formation of definite structures.

HISTOGENESIS DURING REGENERATION.

After removal of the piece the surface epithelium spreads out so as to cover the cut area with a layer of epithelium like that covering the embryo, but somewhat thicker than the latter (see Fig. 3, *g*). Stevens (*op. cit.*) gives an excellent account of this process in *Planaria lugubris*. Direct cell division restores the epithelium to normal form. The cells regenerated on the dorsal surface are not ciliated.

The most active cells in the development of the parenchyma of the new part are cells which closely resemble the cells playing a similar part in embryonic development (Fig. 4, *c*, *k*, *l*) and, like them, they divide by indirect division. These cells probably represent the "Stammzellen" mentioned by Keller.[1] The many branched connective-tissue cells seem also to assume a spherical form and to multiply by indirect division. The new connective tissue (Fig. 4, *m*) resembles the embryonic (Fig. 4, *f*). The new surface musculature may arise from the large cells shown in Fig. 6, *f*, cells which resemble the embryo myoblasts. The musculature of the parenchyma seems, however, to arise from cells of the type shown in Fig. 4, *h* and *l*, as do also the rhabdite cells.

The epithelium lining the intestinal branches which extend into the new parts apparently multiplies by direct division (Fig. 5, *c* and *d*).

Cells bordering immediately upon the intestines multiply by indirect division (Fig. 5, *e*). I am uncertain whether these cells contribute to the supply of the intestinal epithelium, but am inclined to think that they do not. Stevens (*op. cit.*) thinks that they may. The new nervous system arises by direct extension of fibers and apparently also of cells from the old into the new parts. Fig. 7, *c*, represents a growing bundle of cells and fibers of this nature. Fig. 7, *f*, shows direct nuclear division in the vesicular nuclei of the cells which accompany the fibers into the new parts. At present, however, no certain evidence can be offered that parenchyma cells do not also become directly transformed into nerve cells under the influence of the old nerve cords. The majority of investigators believe that the nervous system is differentiated from the parenchyma, though in direct continuity with the old nervous system.[2]

The reproductive organs seem to arise from the cells of the type shown in Fig. 4, *e*. During their development mitosis is

[1] "Die ungeschlechtliche Fortpflanzung der Süsswasser Turbellarien, *Jenaische Zeitschrift f. Naturwissenschaften*, Vol. XXI, p. 370, 1894.

[2] Flexner, *Journal of Morphology*, Vol. XIV., p. 337, 1898. Stevens, *op. cit.* Morgan, "Growth and Regeneration in *Planaria lugubris*, *Archiv für Entwickelungsmechanik*," Vol. XIII., p. 179, 1901. E. Schultz, "Aus dem Gebiete der Regeneration bei Turbellarien," *Zeitschrift für wissenschaftliche Zoölogie*, Vol. LXXII., p. 1, 1902.

active. E. Schultz (*op. cit.*) has given an excellent account of the regeneration of the reproductive organs in *D. lacteum*. Morgan has shown (*op. cit.*) that in *P. lugubris* pieces cut anterior to the old reproductive organs may regenerate them.

An exceptionally good discussion of histogenesis in forms allied to the planarians may be found in the article by Keller (*op. cit.*).

Factors Determining Development.

Having thus considered the origin of the tissues entering into the new parts, we may now consider the factors concerned in the development of the various regions in planarians. Of these regions the most definite and fundamental are the head and pharynx. In the embryo the head is developed at one extremity of the yolk-cavity, the pharynx at the other. Between these two regions the yolk-cavity is converted by the ingrowth of septa into an axial gut with lateral branches. The axial gut grows forward into the developing head and posteriorly it sends two branches into the caudal region which develops behind the pharynx.

In regeneration we likewise find that pharynx and head are developed with certain relations to the intestinal system of the isolated piece. Brought near to one another by muscular contraction isolated intestinal branches become anastomosed into a unit system. By making certain cuts it is possible to have two or more such unit systems developed in a single piece, as shown in Fig. 12, *B*. Head and pharynx are developed in connection with such a unit system only when there is contained within the piece a portion of the central nervous system. This is shown in Fig. 10, where the unit intestinal system of the anterior lateral slip has neither head nor pharynx developed in connection with it. Furthermore, the piece containing the unit system must be above a minimum size. Thus in very short head pieces and short, narrow lateral slips no pharynx may be developed although the piece contain a portion of the original intestinal system and a portion of the original nervous system.

What determines the location of the anlage of the pharynx? In a previous paper (*op. cit.*) I showed that the pharynx arises in a region posterior to that portion of the intestinal system into which, on general bodily contraction, the contents of the whole intestinal system tend to be forced. I called this portion of the intestinal system the area of "least intestinal pressure" but this term is bad because it admits of several interpretations. The area into which the intestinal contents tend, on general contraction, to be forced, and in which the new pharynx arises, is essentially the area of least bodily compression. In *P. maculata* the stimulus to pharyngeal formation is exerted in an antero-posterior direction in this region. Polarity in the piece is determined by the central nervous system. The pharyngeal anlage first appears near the ventral wall of the body.

In the embryo it seems fair to assume that the contents of the yolk-cavity are forced toward the embryo-pharynx on general contraction. Closure of this would cause a stimulus to pharynx formation to be exerted on the body wall comparable to that exerted in regenerative development.

In cross pieces taken from in front of the pharynx and in lateral slips the relation of pharynx to intestines is fairly clear (Fig. 8 and Fig. 11).

In tail pieces the problem is more complicated. When no reproductive organs are present the pressure exerted within the anterior extremities of the posterior intestinal rami by contraction of the piece tends to force the intestinal contents into the anterior median branches of the posterior rami, and thus to give rise to an intestinal area not at first occupied by a single intestinal cavity, or axial gut, but by several distended branches. Posterior to this area pharyngeal formation may be started even before the median branches of the posterior rami have fused to form an axial gut. When, however, a portion of the pharyngeal pocket or the sexual organs are contained in the piece the anterior median branches of the posterior rami are thereby prevented from expanding so that the intestinal contents are forced further forward into the area anterior to the pharyngeal pocket or to the reproductive organs.

The exact nature of the stimulus giving rise to pharyngeal formation is still in the dark, although we can point out the area where it seems to be exerted. It is fairly certain, however, that bodily fluids are forced in considerable quantity into the area of the pharyngeal anlage.

In *Phagocata gracilis* F. R. Lillie[1] has shown that in the pharyngeal anlage a considerable number of pharyngeal pouches are developed and that these subsequently fuse. In lateral slips the pharyngeal pouches on the cut side are formed only subsequently to the development of an intestinal branch on that side. In this animal the pharyngeal area seems to be determined by factors similar in nature to those at play in *Planaria maculata*, but pharyngeal formation in this area is limited not to a single stimulus exerted posteriorly from the region of the axial gut, but, on the contrary, may be excited by stimuli arising from anterior median branches of the chief posterior rami of the developing intestinal system. For a criticism of the view which I have expressed as to the factors determining the seat of pharyngeal formation the reader is referred to Lillie's article.

The pharynx may be formed either near a cut surface or quite within the body of the piece. A new head is formed only in the tissue produced on a cut surface. This surface may be a flat plane. In such a case the median axis of the head will at first be perpendicular to the cut surface. Or the head may be produced at the junction of two planes. Thus, if the anterior end of the worm be removed by a V-shaped cut, the angle of the V pointing posteriorly, and the two sides be allowed to unite, as a rule a new head will be produced, the median axis of which is in line with the chief axis of the worm. If they be not allowed to unite a head will be produced on each side with its median axis perpendicular to the plane of the cut. If the V point anteriorly sometimes a head will be produced on each cut surface; at other times a single head will arise, the median axis of which corresponds with the longitudinal axis of the worm. When a new head arises thus at the juncture of two plane surfaces the median axis of the head seems to bisect the angle formed by the junction of the two cut surfaces. Of course, the cut surfaces may not be planes, but may be very irregular in shape. In all such instances, however, similar axial relations prevail.

The direct stimulus to head and brain formation seems to be due to the presence near the cut surface oi the chief coördi-

[1] "Notes on Regeneration and Regulation in Planarians, *American Journal oj Physiology*, Vol. VI., p. 134, 1901."

nating area of the nervous system of the piece. In most cases of regeneration of a new head, this means the most anterior portion of the old central nervous system. Lillie has stated this point of view very clearly in the article referred to above. But while the stimulus to brain formation arises from the chief coördinating center of the piece, the head and the brain are symmetrically formed about a center of nutrition which is represented by that portion of the intestinal system which lies immediately adjacent to the nerve center, and which is in most direct continuity with the main intestinal apparatus. A symmetrical head is formed both when the stimulus to brain formation comes from one side and when it comes from both sides of this projecting intestinal tip.

There is an interesting correlation between the lateral nerve cords and the eyes. In oblique pieces the most direct stimulus to brain formation comes from that nerve cord which is the more anteriorly situated. As a rule the eye on the side corresponding to this nerve cord is developed earlier, and at first larger, than that upon the other. This is still more marked in the regeneration of oblique cross pieces divided in the median line, as shown by Morgan (*op. cit.* 1901). So, too, in the regeneration of lateral slips the eye on the outer side is usually earlier developed than that on the median.

That stimulus to brain formation depends upon the central nervous system projecting into the area of the cut and that the head is formed symmetrically about the tip of the axial gut, with the cut surface as a base, is indicated by the following experiment. If a longitudinal cut be made lateral to the nerve cord from the region of the head back nearly to the pharynx, and then transversely across the body, the slip thus left attached to the main piece will contain none of the central nervous system of the animal. The slip will contract posteriorly and will bend over so as to become fused to the transverse cut surface. When regeneration is well under way it will be found that the new head is developing on the transverse cut surface, symmetrically about the tip of the old axial gut, as shown in Fig. 10. The contracted anterior slip exhibits no specific influence on the regeneration of the head, although the latter is continuous with it. Subsequently, normal form is usually restored by forward growth of the head region and absorption of the lateral slip. By making two such anterior lateral slips, one on each side, it is possible to get a head to develop posterior to a ring of tissue formed by the anastomosis of these slips. As the head grows it projects dorsally in a forward direction beyond the anastomosing lateral slips.

The influence exerted by the intestinal apparatus upon the regenerating part is probably in the main a nutritive one. Owing to the action of the musculature upon the intestinal system that region of the periphery of the body which receives the richest nutritive supply is probably the area about the tip of the axial gut where the head is differentiated. Owing to its proximity to the axial gut the whole anterior regenerating region also receives a rich nutritive supply and this may account for its rapid growth until normal proportions are reached.

The effect of proximity to the axial gut on regenerating tissue is illustrated by the following experiment.

If a narrow longitudinal slip of tissue be removed from one side of the anterior half of the body and a broad slip from the other, regeneration of tissues will be the more rapid on that side from which the broad slip has been removed. This area of more rapid regeneration lies nearer the axial gut.

Morgan (*op. cit.*, p. 200) gives a picture of an oblique cross piece in which the anlage of a new head has appeared before intestinal anastomosis is visible between the pharyngeal area and the base of the head. Morgan traced the outlines of the intestinal system of the piece from the pigment particles contained within the intestinal cells. I have previously shown, however (*op. cit.*, p. 22), that but little pigment is found in the newly formed anastomotic intestinal branches, so that in the piece mentioned by Morgan an anastomotic axial gut may have existed. By using small, slightly pigmented, starved worms in which the reproductive organs are not developed the intestinal outlines may be fairly accurately followed during life, if the pressure of a cover-glass be used to flatten the specimens. Oblique illumination is often of aid. In the hundreds of generating pieces of planarian which I have examined, I have never seen an instance in which differentiation of a head has taken place otherwise than about the tip of an intestinal branch connected with the main intestinal apparatus of the animal. This intestinal branch may be termed the axial gut because of its subsequent relations to the regenerated worm. Lillie, in a valuable contribution to the subject of regeneration in planarians (*op. cit.*, p. 141), states that "the intestinal system regenerates in relation to the new external parts and not *vice versa*, as maintained by Bardeen; from which it follows that the location of the new parts cannot be due primarily to the form of the gut." As a matter of fact it is probable that mutual relations exist between the intestinal system of the animal and the regenerating parts, each exerting specific influences upon the growth of the other.

After the head and pharynx have been formed, the processes which serve to restore the worm to normal proportions become very marked and, unless certain conditions prevail, such as lack of size or attachment to another individuality (as shown in Fig. 12, *B*), this is soon effected. Bilateral symmetry, however, may arise in partially detached pieces containing neither head nor pharynx. Morgan has made a special and valuable study of the general gross relations borne by an isolated part to the regenerated individual.[1]

In small pieces isolated from sexually mature worms the reproductive organs at first completely disappear, as shown above in discussing regeneration in tail pieces. They are regenerated only after the worm has reached a certain advanced stage of development. In *Planaria maculata*, furthermore, the development of the sexual organs seems to depend upon season. In *Dendrocœlum lacteum* Iijima found that after depositing the cocoons the individuals die, but this is not true of specimens of *P. maculata* kept in captivity. It is probable that there is great variation in different species of planarians in the time of development and the stability of the reproductive organs. Schultze, as mentioned above, gives an excellent account of the regeneration of the reproductive organs in *D. lacteum* (*op. cit.*).

[1] *Op. cit.*, and also "The Internal Influences that Determine the Relative Size of Double Structures in *Planaria lugubris*," BIOLOGICAL BULLETIN, Vol. III., p. 132, 1902.

The destruction of the genital apparatus in isolated tail pieces shows that in the reorganization which takes place during regenerative development highly differentiated tissues, like the musculature of the genitals, are not transformed into new parts, but instead undergo retrograde metamorphosis and serve probably as food stuff for the other tissues. On the other hand, highly organized parts may be utilized directly in the formation of new organs. This is shown by the union of a tip of the pharynx retained in the pharyngeal pocket to a regenerated base.

Summary.

1. The anlage of the pharynx is formed at one extremity of the yolk-cavity of the embryo, that of the head at the other.

2. Up to the period of the formation of the pharynx the yolk-cavity is surrounded by a layer of parenchymal cells and these by a thin epithelial sheet. The parenchymal cells multiply by direct and by indirect division and become reduced in size as they increase in number.

3. The ectodermal cells begin to multiply rapidly by direct division after the pharynx has been formed, and thus give rise to a columnar epithelium. The dorsal cells at no time develop cilia, while the ventral cells produce them before becoming columnar in form.

4. Tissue differentiation begins in the parenchyma shortly before the pharyngeal anlage appears. From the parenchyma are developed intestinal cells, muscle cells, nerve cells, rhabdite cells and gland cells, as well as branched connective-tissue cells and possibly leucocytes. Certain of the parenchyma cells seem to multiply without undergoing further differentiation. Intestinal, nerve and muscle cells multiply by direct division, while the parenchyma cells multiply mainly by indirect division.

5. The central nervous system is first developed in the region of the head and from there the lateral cords grow posteriorly.

6. When removed from the cocoon very young embryos soon die. Slightly older ones die if injured. After the pharynx has become functional the embryos may be cut into two or more parts which will live. Pieces from which the head has been removed will not develop a new head unless they contain well-developed nerve cords.

7. In regeneration the new ectodermal cells arise from the old by direct division. New nerve cells, intestinal cells and muscle cells seem likewise to arise from preëxisting cells by direct division. It is possible, however, that they may spring from the large cells of embryonic type situated in the parenchyma. From the latter the other tissues mainly arise.

8. The new pharynx arises immediately posterior and ventral to the region into which the intestinal contents are forced by general muscular contraction and in response to stimuli arising from this region.

9. A new head is regenerated only in tissue produced at a cut surface, and with definite axial relations to the surface. The direct stimulus to head formation arises from an exposed chief coördinating region of the central nervous system. The head is formed with radial symmetry about the tip of the main intestinal branch extending to the cut surface in the vicinity of the exposed nervous system.

10. After head and pharynx have been differentiated the activities tending to restore the piece to a worm of normal form and proportions become especially marked.

11. During regeneration highly differentiated tissues are destroyed unless they may be directly utilized in the formation of new parts.